THE ESTIMATION OF SALICYLIC ACID IN MILK AND CREAM.

By CECIL REVIS and GEORGE ARTHUR PAYNE.

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Having had recently to estimate this acid, an examination of the existing methods showed us that they are not to be relied on in all circumstances, and the following method, alike applicable to milk and cream, has been worked out by us, and afforded satisfactory results.

The chief causes of error that may arise comprise—

1. The effect of the substance used in precipitating the proteids and fat. For this purpose mercuric nitrate is most generally employed. Made by Wiley's method —from mercury and nitric acid, or from pure mercuric oxide—it is most unsatisfactory, as it removes a large quantity of the salicylic acid.

2. The sparing solubility of salicylic acid causes it to be carried down, when in aqueous solution, by the precipitated proteid if the concentration is relatively high.

3. In the case of cream, the acid seems to be held in solution to a certain extent by the fat.

4. It is not always recognised that in the case of cream the acid may not be completely dissolved, as preservatives are often added in the form of powder.

These difficulties are avoided by the following procedure: Twenty c.c. of the milk, or 20 grams of the cream, are placed in a stoppered flask or weighing bottle holding at least 75 c.c. This is carefully neutralised to litmus paper by adding $\frac{N}{1}$ NaOH, noting the amount thus added. Strict attention must be paid to the neutralisation, as the success of the estimation depends very largely upon it. Forty c.c. of absolute alcohol or rectified spirit (neutral) are added, the flask is closed, and the stopper having been tied down, the flask is placed in hot water (about 95° C.), and shaken at intervals for fifteen minutes. This time must not be shortened or the shaking omitted. The flask is then removed, cooled, and an amount of water necessary to compensate for the proteids and fat added less the amount of $\frac{N}{1}$ NaOH required for the neutralisation. This correction in the case of 20 c.c. of milk may be taken as 2 c.c., but in the case of cream it will vary with the fat percentage.* The contents are poured into a tube and centrifugalised or filtered through a dry filter (rotation is to be preferred). Forty c.c. of the filtrate are placed in a 500 c.c. flask with 100 c.c. of water, with sufficient NaOH solution to render the solution distinctly alkaline, and 60 c.c. distilled off slowly. The residue is washed out into a 250 c.c. flask, and before making up to the mark 2 c.c. of a potassio-mercuric iodide solution are added.† (In the case of cream, 1 c.c. of water is further added to allow for the

* *Corrections for Cream.*—The fat must be estimated approximately:

55	per cent.	fat,	use for	20 grams	12·5 c.c. water.
50	,,	,,	,,	,,	11·4 ,,
40	,,	,,	,,	,,	9·3 ,,
30	,,	,,	,,	,,	7·2 ,,

† *Potassium mercuri-iodide Solution.*—1·35 grams mercuric chloride and 3·32 grams of potassium odide are dissolved in 64 c.c. of water, and 20 c.c. of strong sulphuric acid added.

slight precipitation of proteid.) After a few minutes' standing the solution is passed through a dry filter, and 100 c.c. of the filtrate are extracted three times with about 20 c.c. of ether. The ethereal extracts are mixed in a separating-funnel, washed twice with a few c.c. of water, and then extracted with a solution of caustic soda in the following way: About 20 c.c. of water are added to the ether and a drop of phenolphthalein solution, and then $\frac{N}{10}$ NaOH run in until, on shaking, the red colour of the lower layer is permanent; about 1 c.c. excess is added, and, after well shaking, the lower layer is run off into a 100-c.c. flask; the ether is extracted again with another 20 c.c. of water, to which 1 c.c. of $\frac{N}{10}$ NaOH has been added, and finally with 20 c.c. of water. To the combined extracts in the 100-c.c. flask, a quantity of $\frac{N}{10}$ H_2SO_4, equivalent to the $\frac{N}{10}$ NaOH used, is added, and water to the containing mark. This solution may then be used for the estimation of the salicylic acid in the usual way by the use of iron alum and a standard solution of salicylic acid. There are certain points about this colorimetric process which should be noted. Ammonium iron alum should be used in 1 per cent. solution, and as this solution when used in the strength of 2 c.c. to 50 of the salicylic acid solution rapidly precipitates an insoluble basic substance, the original solution of iron alum should be diluted with about five times its volume of water and evaporated to its original bulk. The filtrate from this no longer precipitates in the test dilution. The test for comparison should, if possible, be adjusted to a concentration of from 0·0005 to 0·001 gram of salicylic acid in 50 c.c., this giving the best depth of colour. Higher concentrations cannot be used, though lower ones present no difficulty. It will sometimes be found that when small quantities of salicylic acid only are present, and the test solution has to be used almost undiluted, that an opalescent solution results on the addition of the iron alum (due to mercury salts derived from the mercuric iodide solution which is dissolved by the ether). In this case the test glass is allowed to stand covered overnight, and in the morning the solution is filtered through a small, dry, hardened filter, when the clear solution can be compared with the standard (no deterioration of colour occurs in this process). If at any time the test solution, though clear, has a reddish tinge, preventing comparison with the standard, the comparison is made by allowing the light to be reflected through a layer of coloured water $\frac{1}{4}$ inch deep, made by adding about 8 c.c. of standard ammonium chloride solution (0·00001 grain per c.c. NH_3) and 1 c.c. Nessler solution to 50 c.c. of water; all difficulty arising from this reddish tinge is thus obviated. The following results illustrate the accuracy of the process:

MILK.

Salicylic Acid in 20 c.c. taken.	Salicylic Acid found.
0·0040 gram	0·0042 gram
0·0160 ,,	0·0157 ,,
0·0401 ,,	0·0387 ,,
0·0802 ,,	0·0757 ,,
0·080 ,,	0·075 ,,
0·080 ,,	0·077 ,,
0·200 ,,	0·187 ,,

CREAM.

In 20 grams taken—

0·020	gram	...	...	...	0·020	gram
0·042	,,	...	...	..	0·043	,,
0·004	,,	...	...	...	0·0042	,,
0·062	,,	...	...	...	0·0626	,,
0·008	,,	...	...	...	0·0079	,,
0·200	,,	...	...	...	0·186	,,

A slight loss seems to take place with amounts of 0·08 gram and above, and is probably due to dilution necessary in the final test. This is negligible with milk, as 0·05 in 20 c.c. is the maximum concentration for salicylic acid, and would never be exceeded. In the case of cream, the experiment should, if necessary, be repeated with 10 instead of 20 grams, though in any case in which the salicylic acid is not in solution erratic results due to sampling must be expected.

As it has been stated that salicylic acid gradually disappears in milk, the point was investigated by this method. A quantity of milk was made up containing 0·081 per cent. of salicylic acid, and the amount estimated after various intervals :

Original solution	...	...	...	...	0·081	per cent.
After 24 hours ...	...	...	...	...	0·082	,,
,, 48 ,, ...	...	...	...	...	0·080	,,
,, 10 days (sample coagulated)			...	...	0·081	,,

Boric acid has no effect on the estimation of the acid; and even benzoic acid, when present to the extent of five times the amount of salicylic acid, does not affect the colour estimation.

The colorimetric method is very accurate and greatly superior to the bromine method.

DISCUSSION.

The CHAIRMAN (Mr. JOHN WHITE) asked whether in the author's experience the addition of salicylic acid to milk or cream was rare or common.

Mr. REVIS said that his experience was confined to supplies which were supposed to be of undoubted purity, and therefore he could not speak from the same point of view as a Public Analyst. The case referred to in the paper was the only one he had met with. He did not think that the use of salicylic acid in milk or cream was at all common at present.

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